AWARD NUMBER: W81XWH-14-1-0497

TITLE: Preventing Cartilage Degeneration in Warfighters by Elucidating Novel Mechanisms Regulating Osteocyte-Mediated Perilacunar Bone Remodeling

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REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
October 2016

2. REPORT TYPE
Annual

3. DATES COVERED
30Sep2015 - 29Sep2016

4. TITLE AND SUBTITLE
Preventing Cartilage Degeneration in Warfighters by Elucidating Novel Mechanisms Regulating Osteocyte-Mediated Perilacunar Bone Remodeling

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0497

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Our overall hypothesis is that an adverse biologic response to protracted high mechanical loads compromises osteocyte-mediated perilacunar remodeling (PLR), bone quality, and cartilage health in post-traumatic osteoarthritis (PTOA). Few molecular details are known about the regulation of PLR or bone quality in healthy bone or in disease. Therefore, we will test the hypothesis that mechanical load and TGFβ signaling interact to regulate PLR, and that this regulation is impaired in, contributes to, and can be targeted for prevention of, the progression of PTOA. We are testing this hypothesis using mouse models and human PTOA tissue. We aim to determine: 1) the extent to which mechanical loading regulates PLR in a TGFβ-dependent manner, 2) the relationship among PLR, strain, TGFβ, and cartilage degeneration, and 3) the causality of PLR in cartilage degeneration. During the first year, we developed and validated new protocols and reagents. During the second year, we found that PLR is TGFβ-regulated, which underlies the deregulation of bone quality in mice with altered TGFβ signaling. Osteocyte-specific deletion of MMP13, a key PLR protease, causes subchondral bone sclerosis as in human PTOA. We also find that PLR is deregulated in human PTOA. We have made great strides in understanding the mechanosensitive regulation of PLR, though a final conclusion requires additional analyses. Since osteocytes have not been implicated in OA, understanding their role in disease has significant potential to yield new drug targets to impede cartilage degeneration.

15. SUBJECT TERMS
Osteocyte, remodeling, bone, bone quality, post-traumatic osteoarthritis, TGF-beta, mechanical load, matrix metalloprotease, perilacunar remodeling, mechanobiology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U Unclassified	U Unclassified	U Unclassified	UU Unclassified	15	

Table of Contents

Page

1. INTRODUCTION

Bone and cartilage cooperate to support healthy joint function – and both tissues are affected by, and contribute to joint disease. However, the mechanisms by which bone defects cause cartilage degeneration, and vice versa, remain unclear. To elucidate these mechanisms, this project focuses on osteocytes in subchondral bone, their ability to support joint health, and their contribution to post-traumatic osteoarthritis (PTOA). Osteocytes sense and respond to mechanical loads, and they are also known to remodel the local bone environment through a process called perilacunar remodeling (PLR). PLR is important for maintaining bone quality. We hypothesize that normally, osteocyte-mediated PLR is mechanosensitive, but that it is impaired by the excessive loads that ultimately drive PTOA. Failure of osteocyte-mediated PLR leads to bone fragility. This fragile bone may be incapable of absorbing the shock of loading that could, in turn, compromise cartilage function and ultimately lead to PTOA. This hypothesis represents an advance beyond what is known because the ability of osteocytes to remodel and strengthen bone has only recently been discovered. Consequently, the possibility of targeting osteocytes with drugs to prevent or treat PTOA has not yet been explored. Therefore, the overall objective of this project is to uncover the effect of mechanical loads on the ability of osteocytes to remodel and strengthen bone. Further, we will determine if defective remodeling causes cartilage degeneration in PTOA. We will accomplish these goals by studying human tissues collected from PTOA veterans who undergo joint replacement surgery, as well as animal models. This approach is powerful because it supports our clear goal of identifying new cellular and molecular targets that can be used therapeutically to prevent or treat PTOA.

2. KEYWORDS

osteocyte, remodeling, bone, bone quality, post-traumatic osteoarthritis, TGF-beta, mechanical load, matrix metalloprotease, perilacunar remodeling, mechanobiology

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major Goals

Aim 1: Determine the extent to which mechanical loading regulates PLR in a TGFβ-dependent manner.

Aim 2: Determine the extent to which PLR defects in human PTOA subchondral bone spatially correlate with strain intensity, TGFβ signaling, or articular cartilage degeneration.

Aim 3: Determine the extent of causality between defective PLR and cartilage degeneration in PTOA.

Year 1-2 Milestones and Percent Completion

- ☑ IACUC Approval – 100% complete
- ☑ CHR Approval – 100% complete
- ☐ Establish the mechanosensitivity of PLR – 60% complete
- ☑ Establish TGFβ-dependence of PLR – 100% complete

☐ Establish the mechanosensitivity of bone quality – 40% complete

☐ Establish the integrity of PLR in PTOA bone – 30% complete

☐ Determine if PTOA uncouples load, TGFβ, PLR – 30% complete

☐ Establish association of cartilage degeneration with PLR – 30% complete

What was accomplished under these goals?

Aim 1: Determine the extent to which mechanical loading regulates PLR in a TGFβ-dependent manner.

Overview: We conducted all of the analyses proposed in Aim 1. As described below, these studies convincingly demonstrate that PLR is regulated by TGFβ in an osteocyte-intrinsic manner, and further, that this regulation is essential for the maintenance of bone quality. The analyses of PLR mechanosensitivity showed the importance of nerve/bone crosstalk in the mechanoregulation of TGFβ signaling, an unexpected but exciting area for continued research.

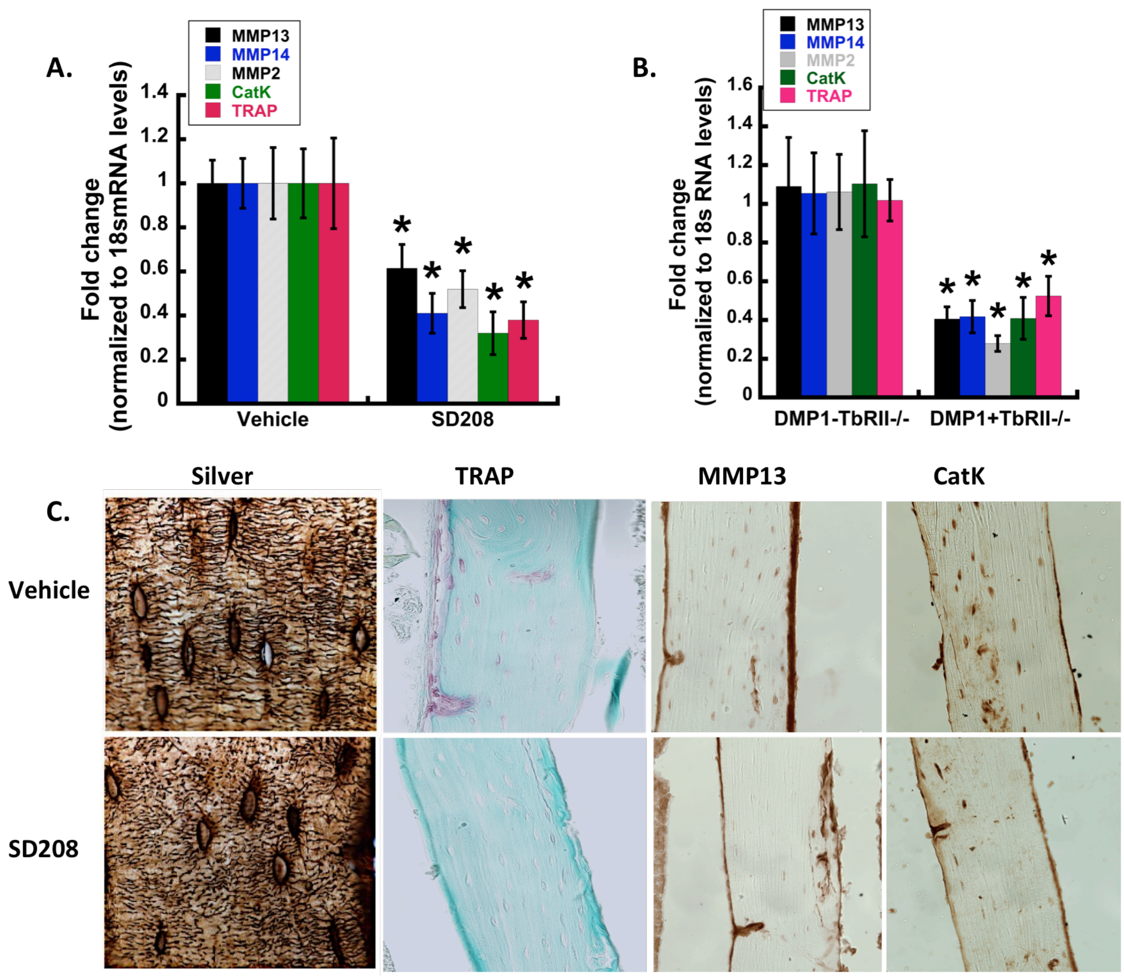


Fig. 1. TGFβ regulates PLR enzymes. Gene expression of MMP13, MMP14, MMP2, Cathepsin K (CatK) and TRAP in long bone of mice administered with vehicle or SD208 (TβRI-inhibitor - 60mg/kg/day) (A) or in DMP1-Cre$^{+/-}$;TβRII$^{fl/fl}$ mice (B), *p<0.05, different from respective control and N=8. Silver staining showing osteocyte canalicular lacunar localization, TRAP staining and IHC for MMP13 and CatK in vehicle and SD208 treated mice. For all, N>3.

TGFβ regulates PLR: Using the same model in which we evaluated the effect of systemic TβRI-inhibition on osteoclasts and osteoblasts (SD-208, Alk5 inhibitor), we investigated the unknown role of TGFβ in osteocytes. After verifying the high bone mass phenotype, we found that systemic inhibition of TβRI drove a strong and coordinated repression in bone of several key PLR enzymes, including MMP2, MMP13, MMP14, CatK and TRAP genes ***(Fig. 1A)***. Immunohistochemistry of cortical femurs revealed a significant decrease in the number of MMP13, MMP14, CatK and TRAP-expressing osteocytes upon TβRI inhibition.

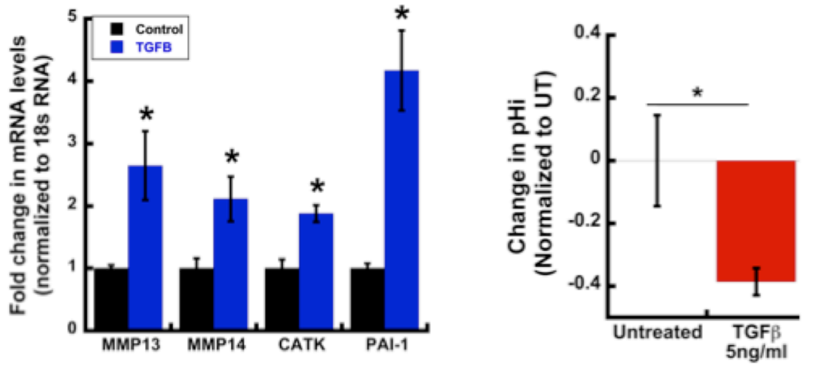


Fig. 2. TGFβ regulates PLR in an osteocyte-intrinsic manner. Left Panel: MLO-Y4 osteocytes treated with TGFβ (5 ng/ml) for 24h showed significantly increased expression of an established TGFβ-inducible gene (PAI1), as well as 3 proteases required for PLR (N=3, *p<0.05). Right Panel: Using MLO-Y4 cells loaded with an intracellular pH-sensitive dye, we found that TGFβ (3 days) reduces intracellular pH, consistent with increased PLR (N=4, *p<0.05).

Given that suppression of these genes has been linked to deregulated PLR by osteocytes, we investigated the regulatory role of TGFβ in PLR. We evaluated hallmarks of PLR, including the osteocyte lacunocanalicular network (LCN), in TβRI-inhibitor and vehicle-treated mice. Impeded TGFβ signaling caused a qualitative decline in the integrity of the osteocyte lacunocanalicular network in the cortical bone ***(Fig. 1B)***. As LCN connectivity is a crucial functional outcome of PLR, our results suggest that TGFβ signaling is an essential regulator of PLR, directly in osteocytes and/or indirectly through interactions with other cells.

To determine if the effect of TGFβ on PLR is osteocyte-intrinsic, we analyzed *in vitro* PLR outcomes in MLO-Y4 cells ***(Fig. 2)***. TGFβ significantly induced the expression of PLR genes, including MMP13, MMP14 and CatK ***(Fig. 2, Left)***. Using a newly developed assay in our laboratory, we found that TGFβ also caused a drop in intracellular pH, a functional measure of osteocyte PLR ***(Fig. 2, Right)***. This decrease in intracellular pH supports bone resorption by osteocytes engaged in PLR, just as it does for osteoclast-mediated bone resorption.

To further evaluate the role of TGFβ in PLR *in vivo*, we developed and evaluated a novel model of osteocyte-specific ablated TGFβ signaling by crossing DMP1-Cre and TβRII flox/flox mice (TβRII$^{ocy-/-}$). These mice exhibit a significant increase in

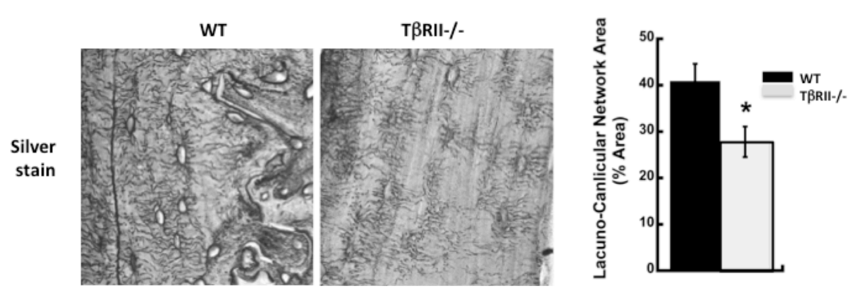


Fig. 3. Osteocyte-intrinsic TGFβ maintains canalicular networks. Lacuno-canalicular network integrity, visualized in histological sections of demineralized bone using a modified silver stain, shows severe canalicular degradation and loss of canalicular area when osteocyte specific TGFβ signaling is blocked in DMP1-Cre$^{+/-}$;TβRII$^{fl/fl}$ mice (N=6 mice/group, *p<0.05).

trabecular bone volume, but no change in cortical thickness or geometry. Immunohistochemistry demonstrated near complete loss of TβRII in osteocytes, but intact TβRII in osteoblasts and other cell types. In addition to a high trabecular bone mass bone phenotype, TβRII$^{ocy-/-}$ bones express dramatically lower levels of MMP2, MMP13, MMP14 and CatK mRNA compared to the control mice ***(Fig. 1B)***. Immunohistochemical staining for MMP13 and MMP14 confirmed this finding. Moreover, degeneration in osteocyte LCN integrity and area was even more pronounced in the TβRII$^{ocy-/-}$ cortical bone than in mice treated systemically with TβRI inhibitors ***(Fig. 3)***. Together, these findings indicate that TGFβ regulates PLR through osteocyte-intrinsic mechanisms.

TGFβ regulates bone quality in an osteocyte-intrinsic manner: We previously showed that TGFβ regulates bone quality, though the cellular mechanism remains unclear. Therefore, we sought to determine if loss of osteocyte-intrinsic TGFβ signaling is sufficient to impact bone quality ***(Fig. 4)***. Flexural strength tests showed that the bending modulus for the animals with osteocyte-specific TβRII deletion was 26.7% lower than in wildtype animals, respectively (n=5, $p<0.05$). Yield strength is also significantly reduced by 27.5% in TβRII$^{ocy-/-}$ bone compared to controls. Therefore, macromechanical behavior of the TβRII$^{ocy-/-}$ bone is impaired, even with no changes in cortical bone mass or geometry.

To further evaluate the role of osteocyte-intrinsic TGFβ signaling in bone quality, we utilized in situ scanning electron microscopy (SEM) fracture toughness tests. Using this approach, we monitor step-wise progression of crack propagation qualitatively and quantitatively. Particularly in the WT bone, we observed a number of toughening mechanisms including crack branching, crack deflection, and uncracked ligament bridging ***(Fig. 4, Top Right)***. However, many fewer instances of each were observed in TβRII$^{ocy-/-}$ bone, in which cracks were much more likely to progress in an unchecked linear manner ***(Fig. 4, Lower, Right)***. TβRII$^{ocy-/-}$ bone has a significantly reduced crack-growth toughness (slope of R-curves), indicating a severe deregulation of bone matrix material properties in mice with an osteocyte-intrinsic defect in TGFβ signaling.

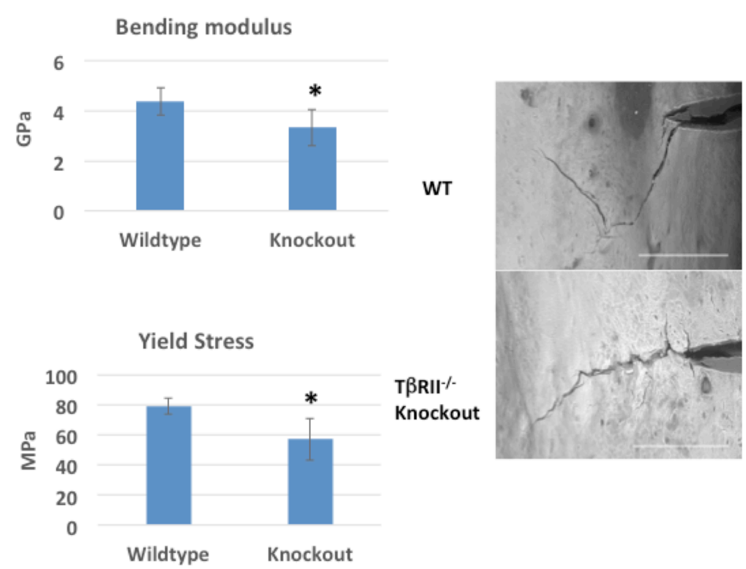


Fig. 4. Osteocyte specific deletion of TGFβ causes a decline in bone quality. Left: In three point bending flexural strength tests, DMP1-Cre$^{+/-}$;TβRII$^{fl/fl}$ bone had significantly reduced bending modulus and yield stress. Right: Using in situ fracture toughness tests, crack extension in wild type bone is accompanied by crack branching and uncracked ligament bridging, whereas these mechanisms are absent in mice with an osteocyte-specific deletion of TβRII, which also show a significant reduction in fracture toughness (N=5 mice/group, *$p<0.05$).

To evaluate the material properties of TβRII$^{ocy-/-}$ bone at a smaller length scale, we used nanoindenation. Even in a preliminary analysis, we detect a significant decrease in bone matrix elastic modulus in TβRII$^{ocy-/-}$ bone relative to wild-type littermates. Analysis of additional specimens is underway and will be completed shortly. This

drop in elastic modulus can be attributed, in part, to reduced bone matrix mineralization. Using both microCT and x-ray tomographic microscopy, we evaluated the mineralization of bone matrix and find that the mineral density of TβRII$^{ocy-/-}$ bone matrix is significantly lower than wild type. Collectively, these results indicate that osteocyte-mediated PLR is essential for the control of bone matrix quality. This work also identifies a novel function for TGFβ in bone, such that osteocyte-intrinsic TGFβ regulates bone quality through PLR. A manuscript detailing these findings is in preparation.

Mechanoregulation of TGFβ is dependent on adrenergic receptor activity: One of the challenges we encountered in the analysis of PLR mechanosensitivity was a site-specific variation in the effect of load on TGFβ activity. Specifically, using mice that express a reporter under control of the TGFβ-responsive Smad binding element (SBE-Luc), we found that load increases TGFβ signaling in the proximal tibia, but repressed it in the middle of the tibia. This spatial regulation of TGFβ mechanosensitivity complicated molecular analyses of whole bone. Unlike in prior studies, the load-dependent differences in TGFβ outcomes, such as Western analysis of phospho-Smad3 levels, yielded variable and ambiguous results. Likewise, we detected no significant differences in PLR outcomes in response to load in these conditions.

We conducted an extremely thorough analysis of multiple experimental variables (loading regimen, mouse age, time course analysis) to better define the conditions for mechanoregulation of TGFβ signaling in order to facilitate our analysis of PLR. This process surprisingly revealed the impact on our results of an anesthetic recovery agent (atipamezole), an antagonist of α-adrenergic receptors. We did not use atipamezole in our original studies (Nguyen, 2013), but began using it in 2014 to facilitate mouse recovery from anesthesia during the in vivo loading procedure. We performed controlled studies in the presence or absence of atipamezole to determine its effect on TGFβ mechanosensitivity.

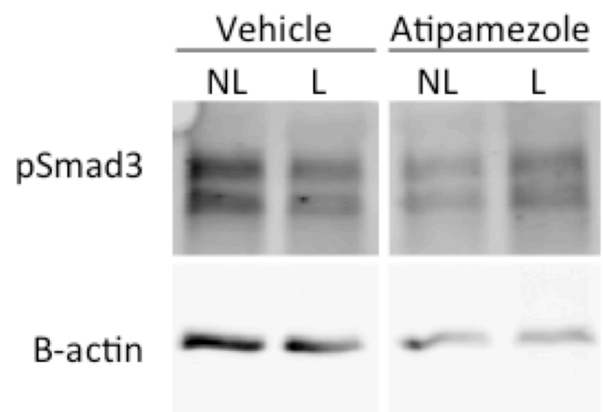


Fig. 5. Mechanoregulation of TGFβ depends on adrenergic receptor activity. Consistent with our prior report (Nguyen, 2013), protein harvested from bone 4h after 10 min of in vivo hindlimb loading shows a reduction in phospho-Smad3, a marker of TGFβ signaling, in the loaded limb (L) relative to the non-loaded contralateral limb (NL). In contrast, mice treated with atipamezole, an adrenergic receptor antagonist, do not show mechano-sensitive repression of TGFβ (N>5/group). Western analysis of β-actin is used to control for protein levels.

Consistent with our original studies, mechanical load represses phospho-Smad3 levels rapidly after tibial loading in the absence of atipamezole ***(Fig. 5)***. However, in the presence of atipamezole, phospho-Smad3 levels are increased or unchanged by load. We have also repeated luciferase imaging of SBE-Luc mice following load in the presence or absence of atipamezole. Preliminary data using this approach further supports the effect of adrenergic receptor activity on TGFβ mechanosensitivity.

Based on this unexpected finding, we are now repeating the analyses proposed in Aim 1 in the absence of atipamezole to evaluate the effect of load on PLR, and the TGFβ dependence of this effect. In addition, we are developing new studies to more fully

investigate the mechanism of adrenergic receptor activity on TGFβ signaling and the nerve/bone interaction.

Aim 2. Determine the extent to which PLR defects in human PTOA subchondral bone spatially correlate with strain intensity, TGFβ signaling, or articular cartilage degeneration.

As detailed in the Year 1 Annual Report, Aim 2 was delayed because our use of surgical waste specimens was not exempt (as proposed) but required CHR approval. Throughout Year 2, we completed the Year 1 pilot analysis of these samples and now have a robust series of PLR protocols for human surgical specimens. Now, we are routinely collecting these samples and continue our analysis to address the questions posed in this Aim. We are on track to complete this analysis within the coming year.

So far, the preliminary analyses confirm the hypothesized relationship between cartilage degeneration in human PTOA, thickened subchondral bone, and deregulated PLR. In areas of maximal cartilage degeneration, canalicular networks are diminished and MMP13 expression is altered. From these initial studies, it is clear that this approach will allow us to answer our questions about the relationship of PTOA and PLR within the coming year.

Aim 3. Determine the extent of causality between defective PLR and cartilage degeneration in PTOA.

A role for PLR in bone sclerosis: Published and preliminary data from our group and others shows the importance of TGFβ and MMP13 in the development of subchondral bone sclerosis in TMJD, osteoarthritis and osteonecrosis. However, the role of osteocyte-mediated PLR in sclerosis was unknown. We found that osteocyte-intrinsic MMP13-deficiency significantly increases trabecular bone volume, which supports a new role for MMP13-dependent PLR in the control of subchondral bone sclerosis. We continue to characterize the baseline phenotype of the DMP1-Cre;MMP13fl/fl bone, as we prepare for the MLI experiments. We will need to hire a new post-doctoral scientist to complete this part of the study since Dr. Fowler recently accepted a position as an independent investigator at another institution. Nonetheless, the mice are now available and we are prepared to pursue these studies over the coming year.

What opportunities for training and professional development has the project provided?

The project has provided training opportunities for Dr. Tristan Fowler, Dr. Claire Acevedo, and JJ Woo. Dr. Fowler has advanced to an independent scientist position at Surrozen, a biotech company that is developing new therapies targeting the Wnt pathway. Dr. Acevedo has presented her research at 2 national meetings and has successfully applied several approaches in materials science and engineering to elucidate the regulation of PLR and bone quality by load or TGFβ signaling. In addition, she has earned independent fellowship funding to support her continued research. JJ Woo has learned several new approaches including histologic analyses of PLR, and handling and analysis of human surgical samples. Over the coming year, Acevedo and Woo will assist in the preparation of abstracts for national meetings and manuscripts for publication. Throughout the course of the project, Fowler, Acevedo, and Woo have mentored rotating graduate, undergraduate, and medical students, as well as residents in orthopaedic surgery and otolaryngology. This project has

provided a professional development opportunity for the trainees in my lab, as well as for those they mentored.

How were the results disseminated to communities of interest?

This work was presented at the 2015 ORS as a talk, at the 2016 ASBMR as a plenary poster, and three abstracts were submitted for the 2016 ORS and we are awaiting news of their acceptance. The submitted ORS abstracts are entitled:

Glucocorticoids suppress osteocyte maintenance of the lacunocanalicular network and bone quality to cause osteonecrosis

Novel role of TGFβ in osteocytes: Regulation of perilacunar remodeling and bone quality

TGFβ regulates fracture toughness via osteocytes

What do you plan to do during the next reporting period to accomplish the goals?

We will complete the remaining milestones from Year 2, as well as those from Year 3:

☐ Establish the mechanosensitivity of PLR
☐ Establish the mechanosensitivity of bone quality
☐ Establish the integrity of PLR in PTOA bone
☐ Determine if PTOA uncouples loading, TGFβ, and PLR
☐ Establish whether cartilage degeneration occurs in areas of disrupted PLR
☐ Establish the extent to which PTOA disrupts PLR
☐ Establish the extent to which PLR defects cause PTOA

To accomplish these milestones, we will continue with the ongoing histologic and molecular studies of PLR mechanosensitivity as proposed in Aim 1, but with different anesthesia agents that do not interfere with adrenergic receptor activity. We will continue the corresponding analyses of bone quality. We will continue to collect and process the human cadaveric and surgical specimens for Aim 2, including the finite element analyses. For Aim 3, we will proceed with the MCI injury studies upon hiring and training of a new post-doctoral scientist to replace Dr. Fowler. In addition, we intend to prepare multiple manuscripts describing our findings.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The greatest impact of our work at the completion of the second year is discovery that PLR is TGFβ-regulated and that deregulation of osteocyte-intrinsic TGFβ signaling is a pivotal mechanism in bone fragility. Since at least half of fractures are not accounted for by low bone mineral density/bone mass, this mechanism may elucidate new therapeutic targets for the treatment of bone fragility due to bone quality defects.

The advances in the past year provide us with a solid foundation to answer the scientific questions posed by this project within Year 3. The impact of understanding the mechanoregulation of PLR, as well as understanding the potential to regulate bone quality, is of pivotal importance, both for understanding the control of normal bone as well as the mechanisms that are deregulated in post-traumatic osteoarthritis.

What was the impact on other disciplines? None

What was the impact on technology transfer? None

What was the impact on society beyond science and technology? None

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

As described above, we had a significant breakthrough late this summer in understanding that the effect of load on TGFβ signaling depends on the activity of adrenergic receptor signaling, presumably due to interaction of peripheral nerve with osteocytes in bone. This is a very exciting, but unanticipated, discovery. While we have learned a great deal as we conducted the proposed experiments, it has delayed our ability to draw final conclusions on the question we originally posed. We will need to redo many of the studies proposed in Aim 1 using a different set of anesthetic agents that do not interfere with adrenergic receptor signaling to determine the mechanosensitivity of PLR. These studies are currently underway but will take approximately 6 months to complete. Nonetheless, the discovery of the role of nerve in regulating the effect of mechanical load on TGFβ signaling could play a vital role in the progression of PTOA. We will write a new grant proposal to explore this idea further.

Actual or anticipated problems or delays and actions or plans to resolve them

Dr. Fowler has accepted a new position. We are in the process of hiring a new post-doctoral scientist to take over the remaining part of his studies.

Changes that had a significant impact on expenditures

Year 1 was under budget due to the unexpected departure of a staff member. We are now spending at the appropriate rate at the end of Year 2.

The increased number of human specimens to be analyzed (12 instead of 8) due to the high tissue variability in our pilot analyses will require increased expenditures above what was budgeted in the project.

The unexpected role of nerve/bone crosstalk in the mechanoregulation of TGFβ signaling requires that we repeat some of the studies in Aim 1 with a different anesthetic agent. This will require additional mice, time, and expenditure.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

The unexpected role of nerve/bone crosstalk in the mechanoregulation of TGFβ signaling requires that we repeat some of the studies in Aim 1 with a different anesthetic agent. This will require additional mice, time, and expenditure.

6. PRODUCTS

Publications, conference papers, and presentations:

The PI was invited to moderate a session at the Gordon Research Conference and to speak on the topic of osteocyte-perilacunar remodeling at the *AAOS/ORS Research Symposium on Joint Crosstalk* in Rosemont, IL in April, 2016. Dr. Alliston also organized this symposium and invited speakers to discuss all aspects of joint crosstalk, including in PTOA. This symposium explored the state of current knowledge and outstanding questions on the topic of joint disease. Other presentations include:

1. Fowler T, Hall-Glenn F, Fields A, Bale H, Ritchie RO, Vail T, Lotz J, **Alliston T.** Osteocyte-Driven Perilacunar Remodeling is Impaired in Glucocorticoid Induced Osteonecrosis. 2015 American Society for Bone and Mineral Research Annual Meeting, Paper MO0239. Seattle, WA.
2. Fowler T, Acevedo C, Mazur C, Hall-Glenn F, Fields A, Bale H, Ritchie RO, Lotz J, Vail T, **Alliston T.** Osteocyte-Driven Perilacunar Remodeling is Impaired in Glucocorticoid Induced Osteonecrosis. 2016 American Society for Bone and Mineral Research Annual Meeting, Paper FR0182. Altanta, GA.

We have one manuscript related to this project in review:

1. Fowler TW, Acevedo C, Mazur CM, Hall-Glenn F, Fields AJ, Bale HA, Ritchie RO, Lotz JC, Vail TP, **Alliston T.** Glucocorticoids suppress osteocyte maintenance of the lacunocanalicular network and bone quality to cause osteonecrosis. 2016 In Review.

We anticipate the submission of multiple manuscripts related to this project in the final year of the grant.

Website(s) or other Internet site(s): None

Technologies or techniques:

We have continued to work on the optimization of PLR outcomes and are developing a manuscript on PLR methods for submission in the coming year.

Inventions, patent applications, and/or licenses: None

Other Products: None

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Only individuals who have contributed more than 1 person month per year are listed.

Name:	Tamara Alliston
Project Role:	PI
Nearest Person Month Worked:	1
Contribution to Project:	Lead the project.
Funding Support:	DOD, NIH, UCSF, NSF

Name:	Tristan Fowler
Project Role:	Post-doctoral Scientist
Nearest Person Month Worked:	12
Contribution to Project:	Perform experiments on PLR.
Funding Support:	DOD

Name:	Claire Acevedo
Project Role:	Post-doctoral Scientist
Nearest Person Month Worked:	5
Contribution to Project:	Perform experiments on bone quality.
Funding Support:	DOD, NIH

Name:	JJ Woo
Project Role:	SRA I
Nearest Person Month Worked:	2
Contribution to Project:	Manage mice, human specimen research.
Funding Support:	DOD, NIH

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Tamara Alliston
Although the PI's effort on the DOD project (1.2 mos) has not changed, the PI's role on the following projects has concluded due to the end of the grants:

NIH R21: The Mechanobiology of TGF-beta Signaling in Chondrocytes (1.8 mos)

The PI has new roles due to the award of these grants, none of which overlaps with the current project:

NSF: Mechanoregulation of growth factor receptor heteromerization and signaling (0.6 mos)

Jeffrey Lotz
No Changes.

Thomas Vail
No Changes.

Alfred Kuo
No Changes.

Alexis Dang
No Changes.

What other organizations were involved as partners?

Organization Name: San Francisco VA Medical Center

Location of Organization: 4150 Clement Street

Partner's Contribution:

Facilities:	Surgical specimens are collected at the SFVAMC Hospital
Collaboration:	SFVAMC staff, Alfred Kuo and Alexis Dang, are collaborators.

8. SPECIAL REPORTING REQUIREMENTS

Collaborative Awards: Not Applicable

Quad Charts: Please see Appendix 1.

9. APPEDICES

Appendix 1: Quad Chart for Year 2

Preventing Cartilage Degeneration in Warfighters by Elucidating Novel Mechanisms Regulating Osteocyte-Mediated Perilacunar Bone Remodeling (PLR)

OR130191

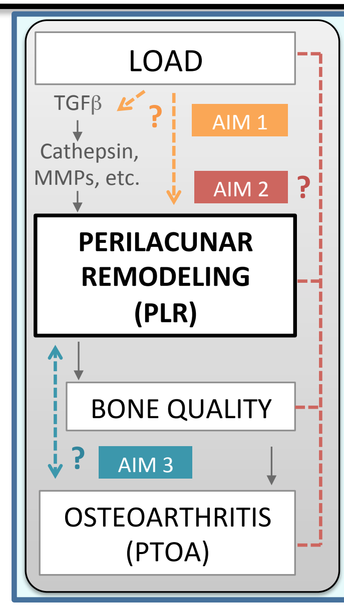
PI: Alliston **Org:** UCSF **Award Amount:** $500,000

Study Aims

- Determine the extent to which mechanical loading regulates PLR in a TGFβ-dependent manner.
- Determine the extent to which PLR defects in human PTOA subchondral bone spatially correlate with strain intensity, TGFβ signaling, or articular cartilage degeneration.
- Determine the extent of causality between defective PLR and cartilage degeneration in PTOA.

Approach

We hypothesize that mechanical load and TGFβ regulate PLR, and further, this regulation is impaired in and contributes to PTOA. To test this hypothesis, perilacunar bone remodeling (PLR) will be examined in genetically modified mice following one-limb loading, TGFβ-inhibition, or traumatic joint injury. To relate these findings to human PTOA, PLR will also be evaluated in human knee specimens recovered following total joint replacement surgery. The effect of defective PLR on cartilage will be evaluated in human and murine specimens.



Accomplishments this quarter:

1. Additional data further strengthening the conclusion that PLR is TGF-beta regulated.
2. Analysis of human PTOA tibial plateaus show spatial differences in PLR outcome measures; namely MMP13 and collagen staining from healthy to diseased regions.
3. DMP1-Cre$^{+/-}$;MMP13$^{fl/fl}$ mice exhibit subchondral bone sclerosis that presents early in life and continues in aging mice, similar to that seen in human PTOA.
4. Significant differences in bone quality observed due to deregulation of TGFβ and MMP13 in osteocytes.
5. Progress in collection in analysis of human specimens

Timeline and Cost

Activities CY	9/14–8/15	9/15-8/16	9/16-8/17
Determine the mechanosensitivity of PLR and PLR proteins			
Determine if TGFβ regulates PLR and is required for its mechanosensitivity			
Determine the effect of load and TβRI-I on perilacunar mineral and material properties			
Determine if regulation of PLR and TGFβ in human subchondral bone is lost in PTOA			
Determine the effect of PLR on bone quality in PTOA			
Determine if PTOA causes PLR defects and if PLR defects cause PTOA			
Estimated Budget (**$500,000 direct**)	**$168,151**	**$190,281**	**$141,568**

Updated: 10/29/16

Milestones

CY14/15 Milestones – studies approved and underway

☑ Local IACUC Approval
☑ CHR Approval

CY15/16 Milestones – investigate mechanisms regulating PLR

☐ Establish mechanosensitivity of PLR
☑ Establish TGFβ-dependence of PLR
☐ Establish the mechanosenstivity of bone quality

CY16/17 Milestones – determine the causality of PLR and PTOA

☐ Establish the integrity of PLR in PTOA bone
☐ Determine if PTOA uncouples loading, TGFβ, and PLR
☐ Establish whether cartilage degeneration occurs in areas of disrupted PLR
☐ Establish the extent to which PTOA disrupts PLR
☐ Establish the extent to which PLR defects cause PTOA

Comments/Challenges/Issues/Concerns

None.

Budget Expenditure to Date

Projected Expenditure: $361,712 out of $500,000
Actual Expenditure: $346,253 out of $500,000